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Eosinophil counts in children with newly diagnosed celiac disease: Is there any association between celiac disease and eosinophil counts?

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Abstract

Aim: In this study, we aimed to evaluate whether there is a relationship between celiac disease (CD) and eosinophils.

Material and Methods: Patients diagnosed with CD under the age of 18 were included in the study. Children who underwent gastroduodenoscopy for any reason and had no abnormalities detected in their biopsies were included in the control group.

Results: Of the 72 patients with CD, 41 were girls, and their mean age was 8.50±4.24 years. Patients with CD had increased eosinophil counts in biopsies taken from the duodenal bulb and the second part of the duodenum. There was no statistically significant difference between the patients and control groups ($p>0.05$). Using the Marsh criteria, three groups of CD patients were created. There were 36 patients (50.00%) in Marsh 3a, 24 (33.33%) in Marsh 3b, and 12 (16.66%) in Marsh 3c. When the peripheral eosinophil counts and eosinophil counts in biopsies of all three groups were compared, no statistically significant difference was discovered ($p>0.05$).

Discussion: We found a higher number of eosinophils in the peripheral blood, the second part of the duodenum biopsy, and the duodenal bulb biopsy of patients with CD in the current study. But we did not detect a statistically significant difference between the patient and control groups. This may be due to the cross-sectional nature of the study and the limited sample size. Our hypothesis that eosinophils may be involved in the pathophysiology of CD needs to be confirmed by larger case series studies.

Keywords

Celiac Disease, Children, Eosinophil, Gastroduodenoscopy, Intestinal Biopsy

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Introduction

Gluten consumption in genetically predisposed people causes the systemic autoimmune disease known as celiac disease (CD), which is marked by a variety of clinical symptoms and malabsorption [1]. The pathophysiology of CD is influenced by gluten consumption, genetic susceptibility, and gluten-induced proinflammatory innate and inappropriate adaptive immune responses. Additionally, it is assumed that some immune system elements (neutrophils, eosinophils, or mast cells) play a role in the pathophysiology of the disease [1,2].

Eosinophil cells, immune system effectors, are found in the peripheral blood and in different tissues of several organs [3]. In healthy individuals, eosinophils are found at high rates in the lamina propria of the gastrointestinal (GI) system mucosa under physiological conditions, except for the esophagus [4]. Numerous medical diseases affecting the GI tract are associated with significant increases in cell numbers and activity [5]. Eosinophils in the GI mucosa that are involved in the immune system have been shown to play a major role in primary eosinophil-associated GI disorders, celiac disease, inflammatory bowel disease, and in host defense against parasite infection and dietary allergens, and their numbers increase when intestinal inflammation is present [6-8]. Eosinophils interact with other immune cells and store biologically active mediators in their granules [9]. They may disrupt the function of the intestinal barrier by increasing the permeability of direct mucosal cell damage as a result of interaction with other cells and increase permeability in the small intestine and colon [10]. It is well known that eosinophils are gastrointestinal immune system resident cells that are essential to the host's defense, especially when helminths and bacterial infections are present [11,12].

The finding of eosinophils in the GI tract in recent decades has raised questions about their function in GI health and disease [13]. Recent information on the pathophysiology of eosinophils clearly shows that the eosinophil is a multifunctional leukocyte, capable of interacting with other immune cells at the border between innate and adaptive immunity [8,14].

Mild duodenal eosinophilic infiltration has been described in patients with CD and severe mucosal atrophy, and it is believed that these cells could be involved in mucosal inflammation [8,15]. Few studies have examined eosinophil levels and the relationship between CD and eosinophils in young CD patients [15-17]. The aim of this study was to evaluate whether there is a relationship between CD and eosinophils.

Material and Methods

This study was performed in the Clinic of Pediatric Gastroenterology between February 2017 and June 2019. The study was executed following the Declaration of Helsinki and approved by The Local Ethics Committee (Mersin University Clinical Research Ethics Committee, Mersin, Turkey, 27 June 2019/263).

The study patient population included patients diagnosed with CD under the age of 18 and diagnosed with CD by intestinal biopsy, as stated in the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) 2012 guideline [18]. Written informed consent was obtained from the patients and their parents before the upper gastrointestinal endoscopy was performed. Children who underwent gastroduodenoscopy for any reason and had no abnormalities detected in their biopsies were included in the control group. Those with concurrent type 1 diabetes mellitus, autoimmune disease, eosinophilic gastrointestinal disease, allergy, parasitic infection, or systemic disease were excluded from the study.

Histological evaluation of the samples

All duodenal biopsies for CD were histopathologically evaluated according to the modified Marsh classification [19].

The samples used for eosinophil count were assessed histologically using a light microscope Nikon Eclipse Ci-L microscope (Nikon, Tokyo, Japan). Regarding the technical aspects, there is a possibility of patchy uptake of eosinophils in healthy and diseased tissues in anatomical subregions. Therefore, the eosinophils in the duodenal lamina propria were counted from 10 randomly chosen non-overlapping high-power fields (HPFs) at a magnification of 400. Subsequently, an average value was established for each case, and eosinophil counts were expressed in mm^2 [20].

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences program version 22.0 (IBM Corporation, Chicago, IL, United States). For the frequencies, percentages, and mean standard deviations (SDs), descriptive statistics were used. The Kolmogorov-Smirnov test was employed to determine whether the data distribution followed a normal pattern. The independent samples t-test was used for nominal data. Using the Mann-Whitney U test, ranges of numerical variables were compared, while the categorical variables were compared using the chi-square test. The Kruskal-Wallis one-way analysis of variance (ANOVA) test was used to compare the three separate groups. A p-value ≤ 0.05 was considered statistically significant.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

Of the 72 patients with CD, 41 were female, with a mean age of 8.50±4.24 years. In the control group, 16 of 26 children were female, with a mean age of 7.77±4.51 years. Age and gender comparisons between the patient and control groups revealed no statistically significant difference ($p>0.05$). The tests revealed a 200 eosinophil (IQR 248.0) count/mm^3 in the peripheral blood of CD patients. Although peripheral blood eosinophil counts were greater in patients with CD than in the control groups, there was no statistically significant difference between the patients and control groups ($p>0.05$). In the control groups, 20 children had a duodenal bulb biopsy. Eosinophil counts were higher in the biopsies from the duodenal bulb, and a second section of the duodenum in patients with CD. Still, there was no statistically significant difference between the patients and control groups ($p>0.05$) (Table 1).

In 55% of the cases, we found an eosinophil count more than 20/HPF in the biopsies from the second part of the duodenum. Using the Marsh criteria, three groups of CD patients were created. There were 36 patients (50.00%) in Marsh 3a, 24 (33.33%) in Marsh 3b, and 12 (16.66%) in Marsh 3c. There was no statistically significant difference in terms of age and gender between the three groups ($p>0.05$). When the peripheral

eosinophil counts and eosinophil counts in biopsies of all three groups were compared, no statistically significant difference was discovered (p>0.05) (Table 2).

Table 1. Demographic and laboratory features of patients and control groups.

	Patients group (n=72)	Control group (n=26)	p
Age	8.50±4.24	7.77±4.51	0.472
Sex (m/f)	31/41	10/16	0.684
WBC (10^5 per mm^3)	7.60±2.07	8.11±2.21	0.307
Eosinophil count (per mm^3) in peripheral blood	200.0 (IQR 248.0)	130.0 (IQR 335.0)	0.923
Duodenal eosinophil count (per mm^2)	44.5 (IQR 77.50)	29.5 (IQR 43.5)	0.098
Eosinophil count in the bulb (per mm^2)	35.5 (IQR 60.80)	20.0* (IQR 44.0)	0.145

*20 children had duodenal bulb biopsy, WBC: White blood cell

Table 2. Comparison of celiac patients according to the Marsh classification.

	Marsh 3a (n=36)	Marsh 3b (n=24)	Marsh 3c (n=12)	p
Age	8.46±4.49	9.30±4.17	7.04±3.38	0.325
Sex (m/f)	16/20	11/13	4/8	0.753
WBC (10^5 per mm^3)	7.35±2.08	7.55±1.72	8.43±2.60	0.300
EOS (per mm^3) in peripheral blood	155.00 (IQR 209.0)	252.50 (IQR 288.0)	200.00 (IQR 162)	0.085
EOS in duodenum (per mm^2)	21.00 (IQR 60.0)	32.00 (IQR 65.0)	45.00 (IQR 50.0)	0.969
EOS in bulb (per mm^2)	31.50 (IQR 68.0)	18.50 (IQR 44.0)	35.50 (IQR 32.0)	0.219

EOS: eosinophil count, WBC: White blood cell

Discussion

In the current study, we detected a higher number of eosinophils in both biopsies from the second part of the duodenum, and duodenal bulb in our patients with CD, but no statistically significant difference was found between the patient and control groups. Peripheral blood eosinophil counts were detected also high in the patient group, but no statistical difference was found between them. Patients with CD were divided into three groups according to the Marsh classification (19). When compared in terms of eosinophil counts in the biopsies of duodenal bulb, and second part of the duodenum, there was no statistically significant difference between these three groups.

Eosinophils were detected at a high rate in gastrointestinal biopsies of patients with inflammatory bowel disease, patients with rheumatoid arthritis, patients with functional dyspepsia, and patients with irritable bowel disease [5,21-23]. It has been suggested that duodenal eosinophils play a role in the pathogenesis of these diseases [5,21-23]. However, their relationship with these diseases remains unclear [5,21-24].

It has been shown that eosinophil numbers are higher in patients with CD [6]. Consistent with these studies, we also found high eosinophil counts in our patients with CD, but no statistical difference was found compared to the control group. In a case series of 150 patients newly diagnosed with CD, eosinophil counts of 3-50/HPF were shown in biopsy specimens. The fact that advanced histological staging of the illness has been linked to mucosal eosinophilia raises the possibility that eosinophils are responsible for the damage to the mucosa [15]. In the present study, a higher number of eosinophils were detected in biopsies in celiac patients compared to the control group. However, there was no statistically significant difference between them. We did not detect any relationship between the histological stages of the disease and the number of eosinophils. We found an eosinophil count of more than 20/HPF in 55% of cases, compared to Brown et al. 's report [15] that this occurred in 25% of cases. This finding may be because our study was cross-sectional and the sample size was small. Consistent with this study, eosinophil counts in biopsy samples of our patients with CD ranged from 0 to 53 /HPF.

It has been shown that eosinophil counts are higher in patients with CD than in healthy children [16,17]. In line with previous investigations, we discovered that patients with CD had higher eosinophil counts than the control group.

In an adult study by Potter et al. [6], a high levels of eosinophils were detected in the duodenum. However, it has been shown that a high rate of eosinophils is not correlated with villous atrophy or clinical symptoms. In parallel with this recent study, we also found high eosinophil counts in patients with CD, that were not correlated with villous atrophy.

Another study found significantly higher eosinophil counts in adult patients with CD whose symptoms persisted despite being on a gluten-free diet [25]. Since the ESPGHAN guideline does not recommend control endoscopy in children with CD, we did not perform this procedure in the follow-up of the patients. Therefore, we could not investigate whether there was any change in eosinophil counts after their gluten-free diet.

To our knowledge, there are few studies on the distribution of eosinophils in the lamina propria of children with CD. In parallel with our study, high counts of eosinophils were detected in some studies [6,16,17]. However, the relationship between this finding and the pathophysiology of CD still remains unclear.

Limitations of the study

First, this study is retrospective. Second, the sample of the control group was limited in size because it was not possible to perform an endoscopy on healthy controls. Third, there are no control biopsies to investigate whether and how eosinophil counts change in celiac patients after responding to a gluten-free diet. The reason for this is that control biopsies are not recommended for patients with CD according to the ESPGHAN guideline, so control biopsies were not obtained after treatment.

Conclusion

We found a higher number of eosinophils in the peripheral blood, second part of the duodenum biopsy, and the duodenal bulb biopsy of patients with CD in the current study. But we did not detect a statistically significant difference between the two groups. This may be due to the cross-sectional nature of the study and the limited sample size. Our hypothesis that eosinophils may be involved in the pathophysiology of CD has to be confirmed by larger case series studies.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest
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